THE UNIVERSITY OF ALBERTA

"THE ROLE OF CARBOHYDRATE IN THE DIET OF THE CHICK"

by

ALBERTA MOREAU ELCOMBE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

SCHOOL OF HOUSEHOLD ECONOMICS

EDMONTON, ALBERTA

FEBRUARY, 1965

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Role of Carbohydrate in the Diet of the Chick" submitted by Alberta Moreau Elcombe in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Experiments were conducted to determine the requirement of the chick for carbohydrate and to study the metabolic effects of feeding "carbohydrate-free" diets in which non-protein energy was supplied by either soybean oil or soybean fatty acids.

Studies showed that the amount of glycerol required for the maximum growth response of chicks fed diets containing 15.4 kcal/gram protein in which non-protein energy was supplied by soybean fatty acids was the amount required for theoretical conversion of fatty acids to triglyceride, i.e. 0.108 grams glycerol per gram of fatty acids. Other studies showed glucose to be at least as effective as glycerol in enabling the chick to utilize fatty acids. The amount of glucose required for maximum growth response has been found to vary from 1/3 to 1 times the amount required for theoretical conversion of fatty acids to triglyceride, i.e. 0.035 to 0.105 grams glucose per gram of fatty acids.

Studies of the metabolic effects of feeding "carbohydrate-free" diets in which non-protein calories were supplied by either soybean oil or soybean fatty acids showed that chicks maintained normal levels of blood glucose and blood lactic acid but showed a marked depression in levels of liver glycogen. Further studies have shown that blood levels of ketone bodies rose when chicks were fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids but remained normal when soybean oil served as the sole source of non-protein energy.

Pair-feeding trials have shown that the growth-stimulating effect of supplementary glucose disappeared when the intake of chicks fed diets containing either 0.035 or 0.210 g glucose/g fatty acids was restricted to that of chicks fed the "carbohydrate-and glycerol-free" diet. The results indicated that under ad libitum feeding, glucose increased growth of chicks fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids by stimulating appetite.

Studies have also shown that chicks fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids utilized nitrogen as efficiently as chicks pair-fed comparable diets containing carbohydrate i.e. 0.035 or 0.210 g glucose/g fatty acids. The results indicated that dietary protein was not diverted from protein to carbohydrate synthesis when "carbohydrate-and glycerol-free" diets containing soybean fatty acids were fed. This indicated either that the dietary protein, i.e. soybean protein, contains glucogenic amino acids in sufficient excess to maintain levels of blood glucose and blood lactic acid in the absence of dietary carbohydrate and glycerol or that chicks can synthesize carbohydrate from fatty acids.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Ruth Renner, Professor of Nutrition, who originated the Research Work being carried on in the Department of Household Economics, and under whose valuable guidance this project was undertaken. Her help and suggestions in the preparation of this manuscript are also gratefully acknowledged. Sincere appreciation is extended to Dr. L. W. McElroy, Head of the Department of Animal Science, for the use of the facilities of its Research Laboratory, as well as to other members of the Faculty and of the Laboratory Research Staff, whose co-operation and encouragement have been much appreciated. Special mention is made, in this regard, of Miss Inez Gordon, Technical Assistant to Dr. Renner, for her invaluable aid at all times. Thanks are also extended to Mr. Paul Larsen for his help in the care of the experimental chicks.

Financial assistance in the form of a grant from the National Research Council is gratefully acknowledged.

TABLE OF CONTENTS

Introduction	Page 1
Part I. Requirement of chickens for carbohydrate	
Literature Review	2
Experiment 1	
Materials and Methods	3
Results and Discussion	7
Experiments 2, 3, 4	
Materials and Methods	9
Results and Discussion	10
Part II. Utilization of Protein by chicks fed "Carbohydrate-and Glycerol-Free" Diets	
Literature Review	14
Experiment 5	
Materials and Methods	16
Results and Discussion	18
Experiment 6	
Materials and Methods	20
Results and Discussion	21
Experiment 7	
Materials and Methods	24
Results and Discussion	27

Part III. Metabolic Effects of feeding "Carbohydrate-Free" and "Carbohydrate-and Glycerol-Free" Diets.

Literature Review

Effect on blood glucose 31

Effect on liver glycogen 32

Effect on blood ketone bodies 33

Materials and Methods 35

Results and Discussion 36

General Discussion 46

Summary 50

Bibliography 52

LIST OF TABLES

Table	Title	Page
1	Composition of high carbohydrate diets	4
2	Composition of diets	6
3	Growth, caloric consumption and utilization of energy when chicks are fed diets containing graded levels of glycerol or glucose	7
4	Composition of diets	9
5	Growth, caloric consumption and utilization of energy when chicks are fed diets containing graded levels of glucose	10
6	Effect of level of glucose on growth and caloric efficiency of chicks	12
7	Compositions of high carbohydrate diets	17
8	Composition of diets	17
9	Effect of level of protein on growth, caloric consumption and caloric efficiency of chicks fed "carbohydrate-free" and "carbohydrate-and glycerol-free" diets	18
10	Effect of glycerol and glucose on growth and protein retention when chicks are pair-fed	21
11	Effect of glycerol and glucose on energy utilization when chicks are pair-fed	23
12	Composition of diets	25

13	Growth and protein retention when chicks are pair-fed diets containing graded levels of glucose	27
14	Efficiency of utilization of energy by chicks pair-fed diets containing graded levels of glucose	30
15	Levels of blood glucose in chicks fed diets containing graded levels of glucose	37
16	Levels of blood lactic acid in chicks fed diets with and without carbohydrate	38
17	Levels of liver glycogen in chicks fed diets with and without carbohydrate	39
18	Levels of blood ketone bodies in chicks fed diets with and without carbohydrate	41
19	Effect of the addition of graded levels of glucose and protein on growth, caloric consumption and blood levels of ketone bodies in chicks fed diets in which non-protein energy is supplied by soybean fatty acids	43
20	Levels of blood ketone bodies in chicks fasted for 24 and 48 hours	44

INTRODUCTION

The carbohydrate requirement of the human and of experimental animals has not been established. Numerous studies have shown that the rat, dog and human can live for extended periods without ingestion of carbohydrate provided sufficient protein or protein and fat is present to meet their energy requirements. Under such dietary regimes, the carbohydrate needs of the body are said to be met by gluconeogenesis from glucogenic amino acids and glycerol. In the case of certain plants and micro-organisms, glucose may also be formed from fatty acids, but in animals the conversion of fatty acids to carbohydrate has not been established.

Recent studies with chicks have shown that fat can completely replace carbohydrate in their diet without altering rate of growth (1). Further studies (2) have shown, however, that when glycerol is removed from a "carbohydrate-free" diet by substituting soybean fatty acids for soybean oil, a marked depression in rate of growth results. This can be overcome to a marked degree by the addition of glycerol in the amount required for theoretical conversion of fatty acids to triglycerides.

The following experiments were undertaken to determine the carbohydrate requirement of the chick, and to study the metabolic effects of feeding "carbohydrate-free" and "carbohydrate-and glycerol-free" diets in which non-protein energy is supplied by soybean oil and soybean fatty acids, respectively.

PART I

REQUIREMENT OF CHICKENS FOR CARBOHYDRATE

Literature Review

Osborne and Mendel (3) in 1924 showed that good growth could be obtained when rats were fed diets virtually free of preformed carbohydrate. The diets contained more than one-half of the energy in the form of fat. Levine and Smith (4) confirmed Osborne and Mendel's work. They found that 86% of the total calories in a "carbohydrate-free" diet could be supplied by fat as long as the protein, mineral and vitamin requirements of the animal were met. Similar results have also been reported by Greisheimer (5) and by Mackay and co-workers (6).

Diets extremely low in carbohydrate have also been consumed by humans. McClellan and DuBois (7) subjected themselves to a relatively "carbohydrate-free" diet for one year by living on a meat diet exclusively, with the amounts of fat and lean meat left to choice. Protein, fat and carbohydrate intakes were 100-140, 200-300 and 7-12 grams per day, respectively. They reported no specific physical changes. Recently, Azar and Bloom (8) reported that consumption of a diet adequate in calories, protein and fat, but lacking carbohydrate for a period of 3 days results in loss of body fat, protein, salt and water, similar to that of a fasting patient.

Chickens have also been shown to tolerate high fat, low carbohydrate diets. Donaldson and co-workers (9) showed that almost complete replacement of carbohydrate by fat in a diet supplying 14.7 kcal/g protein was without adverse effects on growth rate, if nutrient balance was maintained.

Rand, Scott and Kummerow (10) also demonstrated that the chicken's tolerance for fat is essentially unlimited. They found that chicks fed diets supplying 11.5 kcal/g protein grew as rapidly when fat contributed 97% of the non-protein calories as when 30% of the non-protein calories were supplied by fat. More recently, Renner (1) found that fat can completely replace carbohydrate in the diet of the chick without decreasing growth even when protein is limiting in the diet. Further studies in this laboratory (2) have shown, however, that when glycerol is removed from a "carbohydrate-free" diet by substituting soybean fatty acids for soybean oil, a marked depression in growth results. A marked increase in growth rate is produced by the addition of glycerol in the amount needed for theoretical conversion of fatty acids to triglyceride. These results suggest that the ability of the chick to synthesize carbohydrate is limited. The following experiments were conducted, therefore, to study the requirement of the chick for a dietary source of carbohydrate.

EXPERIMENT I

The objects of this experiment were: (1) to determine the requirement of the chick for glycerol when fed "carbohydrate-and glycerol-free" diets, in which non-protein energy is supplied by soybean fatty acids, and (2) to compare the effectiveness of glucose in meeting this requirement.

Materials and Methods

Diets devoid of carbohydrate and carbohydrate and glycerol were formulated from the high carbohydrate diet (Table I) by substituting either soybean oil or soybean fatty acids isocalorically for glucose using the values 3.64, 9.21, and 8.65 kcal/g for the metabolizable energy content of glucose,

soybean oil and soybean fatty acids, respectively. The diets were formulated

Table 1

Composition of high carbohydrate diet

Ingredients	%
Constants	
Soybean protein[1]	23.59
Methionine	.81
Glycine	.63
Soybean oil	2.00
Limestone	1.49
Dicalcium phosphate	1.70
Sodium chloride	.60
Mineral mixture[2]	1.50
Vitamin mixture[2]	.52
Antioxidant[3]	.025
Source of non-protein energy	
Glucose	67.14

1. Promine R - Central Soya Chemurgy Division, Chicago 39, Illinois

2. Mineral and vitamin mixture supply in mg/100g diet: 930 KH_2PO_4, 495 $MgSO_4 \cdot 7H_2O$, 0.29 KI, 28 $FeSO_4 \cdot 7H_2O$, 0.78 $CuSO_4$, 12.5 $ZnCl_2$, 0.17 $CoCl_2 \cdot 6H_2O$, 0.83 $Na_2MoO_4 \cdot 2H_2O$, 0.22 Na_2SeO_3, 37 $MnSO_4 \cdot H_2O$, 1.0 thiamine, 1.0 riboflavin, 4.0 calcium pantothenate, 0.04 biotin, 2.0 pyridoxine, 8.0 niacin, 0.3 folacin, 0.3 menadione, 0.005 vitamin B_{12}, 210 choline chloride, 1000 USP vitamin A, 150 IC units vitamin D_3, 3.31 I U vitamin E, 1 aureomycin.

3. Ethoxyquin

to contain 15.4 kcal/g protein since previous work (1) had shown that, at this level, protein is present in sufficient quantities to promote rapid growth but is not in excess. The caloric density of the "carbohydrate-free" diets was maintained approximately equal to the high carbohydrate diet by

the addition of cellulose.

Diets containing graded levels of glycerol or glucose were formulated from the high carbohydrate diet (Table I) by the isocaloric substitution of mixtures of glycerol and soybean fatty acids or glucose and soybean fatty acids for glucose. Glycerol was assumed to be completely absorbed and to have a metabolizable energy value equal to its gross energy value, of 4.32 kcal/g. The compositions of diets containing 0, 1/3, 2/3 and 3/3 the amount of glycerol or glucose required for theoretical conversion of fatty acids to triglyceride are shown in Table 2.

The soybean fatty acids were prepared by alkaline hydrolysis of crude degummed soybean oil. After acidification, the fatty acids were separated from the acid seat by decantation and water washed to remove acid and glycerol. Traces of water were removed by heating under reduced pressure in an atmosphere of nitrogen.

Each diet was fed to duplicate lots of ten male crossbred (White Plymouth Rock X Columbian White) chicks from 4 days to 4 weeks of age. The chicks were housed in electrically-heated, thermostatically-controlled battery brooders with raised-wire-screen floors, in a temperature-controlled laboratory. The chicks were reared to 4 days of age on the "carbohydrate-free" diet containing soybean oil. They were then allotted on the basis of body weight to the experimental groups and fed the experimental diets to 28 days of age. Feed and water were supplied ad libitum. Data on growth and feed consumption were obtained weekly and feed wastage was determined daily.

Table 2

Composition of diets

Treatment			Constant ingredients	Soybean oil	S.F.A.	Glycerol	Glucose	Cellulose[2]	Total
Source of energy	g/g S.F.A.[1] Glycerol	g/g S.F.A.[1] Glucose	g	g	g	g	g	g	g
Glucose	-	-	32.86	-	-	-	67.14	-	100.00
Soybean oil	-	-	32.86	26.54	-	-	-	7.19	66.59
S.F.A.	-	-	32.86	-	28.25	-	-	7.19	68.30
"	0.036	-	32.86	-	27.75	1.00	-	7.19	68.80
"	0.072	-	32.86	-	27.27	1.98	-	7.19	69.30
"	0.108	-	32.86	-	26.80	2.91	-	7.19	69.76
"	-	0.035	32.86	-	27.84	-	.99	7.19	68.88
"	-	0.070	32.86	-	27.44	-	1.95	7.19	69.44
"	-	0.105	32.86	-	27.04	-	2.88	7.19	69.97

1. Soybean fatty acids

2. Solka Floc, S.W. - 40 - A, Brown Forest Products Ltd, Montreal, Quebec.

Results and Discussion

The experimental plan and results obtained are summarized in Table 3.

Table 3

Growth, caloric consumption and utilization of energy when chicks are fed diets containing graded levels of glycerol or glucose

Treatment			Avg. wt		
Source of energy	g/g S.F.A.[1] Glycerol	Glucose	4 wk. g	Kcal consumed[2]	Kcal consumed /g gain
Glucose	-	-	439[3]	2014	5.17
Soybean Oil	-	-	485	2128	5.00
S.F.A.	-	-	342	1552	5.45
"	0.036	-	368	1693	5.41
"	0.072	-	413	1955	5.45
"	0.108	-	450	2053	5.23
"	-	0.035	432	1985	5.34
"	-	0.070	428	1919	5.30
"	-	0.105	420	1880	5.20

1. Soybean fatty acids.

2. Calculated using calculated metabolizable energy values for the diets.

3. Values are averages of duplicate groups each containing 10 chicks.

Analysis of variance (11) and application of Duncan's multiple range test (12) to the data on growth showed that deletion of glycerol from the "carbohydrate-free" diet by substituting soybean fatty acids for soybean

oil caused a marked decrease in rate of growth. The addition of either 0.072 g glycerol/ g fatty acids or 0.035 g glucose/ g fatty acids significantly ($P < 0.05$) increased rate of growth. The addition of greater amounts of glycerol or glucose did not cause any further significant ($P > 0.05$) increase in rate of growth although the difference between growth of chicks fed 0.072 g glycerol/ g fatty acids and 0.108 g glycerol/ g fatty acids approached significance. Results indicated that when 0.108 g glycerol/ g fatty acids was included, growth of chicks did not differ significantly ($P > 0.05$) from growth of chicks fed soybean oil.

Analysis of variance (11) of the data on caloric efficiency indicated that, in this experiment, chicks utilized energy as efficiently when fed a "carbohydrate-and glycerol-free" diet as when diets containing carbohydrate and glycerol were fed.

The results of this experiment indicated the requirement of the chick for glycerol to be approximately the amount required for theoretical conversion of fatty acids to triglyceride i.e. 0.108 g glycerol/ g fatty acids. Results also showed that glucose was at least as effective as glycerol in promoting growth of chicks fed "carbohydrate-free" diets containing soybean fatty acids.

EXPERIMENTS 2, 3, 4

Results of Experiment 1 indicated that the addition of 0.035 g glucose/ g fatty acids was as effective as the addition of either 0.070 or 0.105 g glucose/ g fatty acids in promoting growth of chicks fed "carbohydrate- and glycerol-free" diets. Experiment 2 was designed to determine the effect

on growth of the addition of less than 0.035 g glucose/ g fatty acids to diets in which non-protein energy was supplied by soybean fatty acids.

Materials and Methods

The compositions of the experimental diets are given in Table 4.

Table 4

Composition of diets

Treatment		Constant ingredients	Soybean oil	S.F.A.	Glucose	Cellulose[2]	Total
Source of energy	Level of glucose g/g S.F.A.[1]	g	g	g	g	g	g
Soybean oil	-	32.86	26.54	-	-	7.19	66.59
S.F.A.	-	32.86	-	28.25	-	7.19	68.30
"	0.0099	32.86	-	28.14	.25	7.19	68.44
"	0.018	32.86	-	28.04	.50	7.19	68.59
"	0.026	32.86	-	27.94	.75	7.19	68.74
"	0.035	32.86	-	27.83	1.00	7.19	68.88
"	0.105	32.86	-	26.99	3.00	7.19	70.04

1. Soybean fatty acids
2. Solka Floc. S. W. - 40 - A, Brown Forest Products Ltd., Montreal, Quebec

The diets were formulated by substituting soybean oil, soybean fatty acids and soybean fatty acids plus glucose isocalorically for glucose in the diet given in Table I.

Each diet was fed to duplicate lots of ten male crossbred chicks from 4 to 28 days of age. The methods of allotment, feeding and housing of the chicks were the same as in Experiment I.

Results and Discussion

Growth, caloric consumption and caloric efficiency of chicks fed diets containing graded levels of glucose are summarized by the data in Table 5.

Table 5

Growth, caloric consumption and utilization of energy when chicks are fed diets containing graded levels of glucose.

Treatment				
Source of energy	Level of glucose g/g S.F.A.[1]	Avg. wt 4 wk g	Kcal consumed[2]	Kcal consumed /g gain
Soybean Oil	-	492[3]	2296	5.15
S. F. A.	-	304	1657	6.49
"	0.0099	307	1633	6.37
"	0.018	335	1734	6.07
"	0.026	361	1790	5.72
"	0.035	379	1905	5.84
"	0.105	437	2070	5.33

1. Soybean fatty acids
2. Calculated using calculated metabolizable energy values for the diets
3. Values are averages of duplicate groups each containing 10 chicks

Analysis of variance (11) and application of Duncan's multiple range test (12) to the growth data showed that the addition of either 0.0099 or 0.018 g glucose/ g fatty acids did not increase growth of chicks significantly ($P > 0.05$). Increasing the level of supplementation from 0 to 0.026 or 0.035 g glucose/ g fatty acids significantly ($P < 0.05$) increased growth rate. The addition of 0.105 g glucose/ g fatty acids resulted in a further increase in rate of growth ($P < 0.05$).

The finding that the addition of 0.035 g glucose/ g fatty acids was less effective in promoting growth than 0.105 g glucose/ g fatty acids was in contrast to results of the preceding experiment which had indicated a similar response at these two levels of supplementation.

Statistical analysis of the data on energy utilization showed that in this experiment chicks fed "carbohydrate-free" diets containing soybean fatty acids utilized energy less efficiently than did chicks fed similar diets containing soybean oil. The addition of 0.105 g glucose/ g fatty acids significantly ($P < 0.05$) increased efficiency of utilization of energy. At this level of supplementation chicks utilized energy as efficiently as chicks fed the diet containing soybean oil ($P > 0.05$). The addition of 0.0099, 0.018, 0.026, or 0.035 g glucose/ g soybean fatty acids, however, was, in each case, ineffective in increasing efficiency of utilization of energy ($P > 0.05$).

The finding that chicks fed "carbohydrate-free" diets containing soybean fatty acids utilized energy less efficiently than chicks fed similar diets containing soybean oil is in contrast to the findings in

Experiment I (Table 3) where utilization was equal on both diets. It should also be noted that in Experiment 2, chicks fed soybean fatty acids grew slower and required more glucose to obtain the maximum growth response than did chicks in Experiment 1, even though control chicks grew at the same rate. Whether these differences in response to glucose were due to differences in the carbohydrate or glycerol contents of the diets, or to variation in soybean fatty acids is unknown.

In two subsequent experiments (3 and 4), the experimental plans and results of which are summarized by the data of Table 6, it was found that the addition of 0.035 g glucose/ g soybean fatty acids was

Table 6

Effect of level of glucose on growth and caloric efficiency of chicks

Treatment		Experiment 3		Experiment 4	
Source of energy	Level of glucose g/g S.F.A.[1]	Avg wt 4 wk g	Kcal /g gain[2]	Avg wt 4 wk g	Kcal / g gain[2]
Glucose	-	447[3]	5.59	514[4]	5.26
Soybean Oil	-	490	5.09	515	4.90
S. F. A.	-	335	5.60	337	5.57
"	0.018	338	5.63	-	-
"	0.035	425	5.22	399	4.97
"	0.070	-	-	395	5.06
"	0.105	400	5.44	400	5.15
"	0.210	460	5.36	423	4.86

1. Soybean fatty acids
2. Calculated using calculated metabolizable energy values for the diets.
3. Values are averages of duplicate groups each containing 10 chicks.
4. Values are averages of triplicate groups each containing 9 chicks.

as effective in promoting growth of chicks as was the addition of anything up to 0.210 g glucose/ g soybean fatty acids.

Analysis of variance (11) of the data on caloric efficiency in Experiment 3 indicated that, as in Experiment 1, chicks fed "carbohydrate-free" diets containing soybean fatty acids utilized energy as efficiently as did chicks fed similar diets supplemented with glucose or in which non-protein energy was supplied by soybean oil. Similar analysis of the data on caloric efficiency in Experiment 4 showed that, as in Experiment 2, chicks fed "carbohydrate-free" diets containing soybean fatty acids utilized energy less efficiently than chicks fed diets containing soybean oil or soybean fatty acids plus graded levels of glucose. In Experiment 4, the addition of 0.035 g glucose/ g fatty acids permitted chicks to utilize energy as efficiently as chicks fed soybean oil.

Statistical treatment of the data in the four experiments indicated that chicks fed diets in which non-protein energy was supplied by soybean fatty acids utilized energy less efficiently than when energy was supplied by soybean oil or soybean fatty acids plus the amount of carbohydrate required for theoretical conversion of fatty acids to triglycerides. Whether the energy was lost as heat or as excretory products awaits further study.

The preceding experiments have shown that the addition of 0.108 g glycerol/ g soybean fatty acids or 0.035 - 0.105 g glucose/ g soybean fatty acids permitted growth to increase from approximately 65% to approximately 85% of that of chicks fed soybean oil. The role which glucose and glycerol play in enabling chicks to grow when fed "carbohydrate-free" diets containing soybean fatty acids forms the basis of the following experiments.

PART II

UTILIZATION OF PROTEIN BY CHICKS FED "CARBOHYDRATE- AND GLYCEROL-FREE" DIETS

LITERATURE REVIEW

In 1876, Wolffberg concluded, from the observation that glycogen was deposited in the livers of fowls first starved and then fed a carbohydrate-free meat powder, that protein could be converted to carbohydrate. Since that time studies in a variety of species of animals have been interpreted as indicating that roughly one-half of an average protein can be converted to carbohydrate (13) (14).

Whether nitrogen retention decreases when calories from carbohydrate are completely replaced by calories from fat is controversial. Munro (15) concluded from a survey of the literature that, in the case of man and the dog, the substitution of fat for carbohydrate caused a deterioration in nitrogen balance, while in the case of the rat he found the evidence to be contradictory. Subsequent studies by Thomson and Munro (16) showed that, when fat was exchanged isocalorically for carbohydrate in the diet of the rat, urinary nitrogen output increased for a few days but that by the end of the fourth day it had returned to its former level. They concluded that the transitory nature of the response may account for the differences in reported findings of investigators studying the response in nitrogen retention in the rat when carbohydrate is replaced by fat.

The chick embryo, developing in a medium containing only

1.0% carbohydrate, conserves protein diverting little from growth to synthesis of glucose precursors. This is evident from comparisons of the protein content of eggs and embryos. Jull (17) reported that the chick embryo stores 98, 82 and 43%, respectively, of the protein, carbohydrate and fat contained in the egg. More recently, Grau (18) found that 93% of the protein in the egg was contained in the chick at hatching.

The ability of the hatched chick to utilize a low carbohydrate diet without diverting amino acids from protein to carbohydrate synthesis has been reported by Rand, Scott and Kummerow (10). They found that chicks fed diets supplying 11 kcal/g protein, in which 97% of the non-protein energy was supplied by fat, utilized protein as efficiently as when non-protein calories were supplied by a mixture of fat and carbohydrate. Recently, Renner (1) showed that even when protein was limiting, chicks fed "carbohydrate-free" diets in which non-protein energy was supplied by triglyceride utilized protein as efficiently as when comparable diets rich in carbohydrate were fed. Recently, Donaldson (19) observed that carbohydrate-fed chicks synthesized more fat than did chicks fed high-fat (no glucose) diets. Since fat synthesis requires energy, he proposes that, at a given body weight, total energy available for protein synthesis could be reduced and nitrogen retention depressed by carbohydrate feeding. Results of nitrogen retention reported by Rand and co-workers (10) and Renner (1) do not support this concept.

In all the foregoing studies, protein utilization has been studied when non-protein calories are provided by triglycerides; similar studies in which non protein energy is supplied by fatty acids have not been

reported. Since there was a possibility that the depression in growth of chicks fed "carbohydrate-and glycerol-free" diets might be due to the diversion of amino acids from growth to carbohydrate synthesis, studies were conducted to determine the effect of supplementary glucose on protein utilization. In addition, studies were conducted to determine the effectiveness of protein as a carbohydrate precursor in the chick.

EXPERIMENT 5

Results of the preceding experiments indicated the requirement of the chick for glucose to range from 0.035 - 0.105 g glucose/g fatty acids when diets containing 15.4 kcal/g protein were fed, in which soybean fatty acids served as the source of non-protein energy. The object of Experiment 5 was to determine whether the requirement for carbohydrate could be met by increasing the protein content of the diet.

Materials and Methods

Diets containing 15.4, 13.2 and 11.0 kcal/g protein and in which non-protein energy was supplied by either soybean oil or soybean fatty acids were formulated from their high carbohydrate counterparts (Table 7) by substituting soybean oil or soybean fatty acids isocalorically for glucose. The composition of the diets fed is given in Table 8.

Each diet was fed to duplicate lots of ten male crossbred chicks from 4 to 28 days of age. This experiment was conducted in conjunction with Experiment 3. The methods of allotment, feeding and housing of the chicks were as in Experiment 1.

Table 7

Composition of high carbohydrate diets

Ingredients	Level of protein, kcal/g protein		
	15.4	13.2	11.0
	g	g	g
Soybean protein[1]	23.59	27.64	33.16
Methionine	0.81	0.95	1.14
Glycine	0.63	0.74	0.89
Soybean oil	2.00	2.00	2.00
Limestone	1.49	1.49	1.49
Dicalcium phosphate	1.70	1.70	1.70
Sodium chloride	0.60	0.60	0.60
Mineral mixture[2]	1.50	1.50	1.50
Vitamin mixture[2]	0.52	0.52	0.52
Antioxidant[3]	0.025	0.025	0.025
Glucose	67.14	62.84	57.07

1. Promine, Central Soya Chemurgy Division, Chicago 39, Illinois.
2. As in Table 1
3. Ethoxyquin.

Table 8

Composition of diets

Treatment						
Kcal/ g protein	Energy source	Constant ingredients	Soybean oil	S.F.A.[1]	Cellulose	Total
		g	g	g	g	g
15.4	Soybean oil	32.86	26.54	--	7.19	66.59
	S.F.A.	32.86	--	28.25	7.19	68.30
13.2	Soybean oil	37.16	24.75	--	6.03	67.94
	S.F.A.	37.16	--	26.35	6.03	69.54
11.0	Soybean oil	43.02	22.31	--	5.13	70.46
	S.F.A.	43.02	--	23.75	5.13	71.90

1. Soybean fatty acids
2. Solka Floc, S.W. -40- A, Brown Forest Products Ltd., Montreal, Quebec

Results and Discussion

Summarized in Table 9 are data showing the four-week weights, caloric consumption and caloric efficiencies of the chicks fed diets containing graded levels of protein and in which non-protein calories were supplied entirely by soybean oil or soybean fatty acids.

Table 9

Effect of level of protein on growth, caloric consumption and caloric efficiency of chicks fed "carbohydrate-free" and "carbohydrate-and glycerol-free" diets.

Treatment				
Kcal/ g protein	Energy source	Avg. wt 4 wks. g	Kcal consumed	Kcal consumed /g gain
15.4	Soybean Oil	490[1]	2207	5.09
	S.F.A.[2]	335	1567	5.59
13.2	Soybean Oil	492	2245	5.11
	S.F.A.	367	1800	5.43
11.0	Soybean Oil	507	2273	5.24
	S.F.A.	414	1922	5.33

1. Values are averages of duplicate groups each containing 10 chicks.
2. Soybean fatty acids.

Analysis of variance (11) and application of Duncan's multiple range test (12) to the data on growth showed that increasing the protein content of diets containing soybean oil did not affect rate of growth; however, when non-protein calories were supplied entirely by soybean fatty acids, chicks grew significantly faster ($P < 0.05$) when fed a diet

containing 11.0 kcal/g protein than when diets containing 13.2 or 15.4 kcal/g protein were fed. Results also showed that, at the three protein levels, chicks fed diets containing soybean fatty acids grew significantly slower than did chicks fed comparable diets containing soybean oil.

Similar statistical treatment of the data on energy utilization showed that level of protein did not significantly ($P > 0.05$) increase efficiency of utilization of energy in either the soybean oil or soybean fatty acid series.

In order to formulate diets containing 13.2 and 11.0 kcal/g protein, 0.162 and 0.372 g extra protein/ g fatty acids, respectively, was added to diets containing 15.4 kcal/g protein. In Experiment 3 (Table 6) which was run concurrently, the addition of 0.035 g glucose/g fatty acids to the diet containing 15.4 kcal/g protein was as effective in promoting chick growth as was the addition of 0.372 g extra protein/g fatty acids. These results indicated that the requirement of the chick for carbohydrate can be met by protein but that protein is only about 1/10 as effective as glucose in meeting this requirement. Studies in other species have indicated that 58% of protein is a potential source of glucose (14).

EXPERIMENT 6

Results of preceding experiments showed that the addition of glucose or extra protein to a "carbohydrate-free" diet in which non-protein energy was supplied by soybean fatty acids increased rate of growth and feed consumption. The object of this experiment was to determine the effect of supplementary glucose on growth and nitrogen retention when feed intake was restricted to that of chicks fed a "glucose-and-glycerol-free" diet.

Materials and Methods

Duplicate groups of 10 male crossbred chicks (White Plymouth Rock X Dominant White) were fed diets containing 15.4 kcal/g protein and in which non-protein energy was supplied by soybean fatty acids, soybean fatty acids + 0.035 g glucose/g fatty acids or soybean oil. The food intakes of chicks receiving diets containing soybean fatty acids + glucose and soybean oil were restricted to that consumed by chicks fed a comparable diet containing soybean fatty acids with no added glucose. Chicks being pair-fed received their daily allotment of feed once every 24 hours.

The composition of the diets fed are as shown in Table 4. The methods of allotment, housing and weighing were identical with those of Experiment 1.

At the termination of the experiment the chicks were killed without loss of blood. After cooling, the contents of the gastro-intestinal tracts were removed and the entire carcasses from each

experimental group were frozen, ground, mixed and an aliquot dried by lyophilization. In order that tissue gains could be determined, a representative lot of chicks was killed at the beginning of the experiment and prepared for analysis in a similar fashion. Carcass samples were analyzed for protein, fat and moisture as described by Hill and Anderson (20).

Results and Discussion

Summarized in Table 10 are data showing the average weights of chicks and the amounts of protein retained when the nutrient intakes of all chicks were maintained equal.

Table 10

Effect of glycerol and glucose on growth and protein retention when chicks are pair-fed.

Source of non-protein energy	Avg. wt 4 wks. g	Protein gain g	Protein intake g	Protein retention[1] %
S.F.A.[2]	322	46.2	116.0	40.6
	286	46.6	107.9	43.2
	304[3]	46.4	112.0	41.9
S.F.A.+ 0.035 g glucose/g F.A.	325	50.8	109.5	46.8
	326	47.6	108.9	44.1
	326	49.2	109.2	45.4
Soybean Oil	358	55.9	112.0	50.0
	366	61.7	113.2	54.8
	362	58.8	112.6	52.4

1. (Gain in carcass nitrogen, g/nitrogen consumed, g) X 100.
2. Soybean fatty acids.
3. Underlined values are averages of duplicate groups.

Analysis of variance (11) of the growth data indicated that when the nutrient intake of chicks fed diets containing soybean fatty acids + glucose or soybean oil was limited to that of chicks fed soybean fatty acids, growth rates did not differ significantly. ($P > 0.05$).

Previous studies showed that the addition of glucose to a "carbohydrate-and glycerol-free" diet caused a marked increase in rate of growth when chicks were fed ad libitum. Since differences in growth rate disappeared when chicks were pair-fed, it appears that glucose stimulated growth by increasing appetite. Whether glucose acted to increase food intake by increasing rate of absorption, by preventing the accumulation of some metabolite in the blood stream or in some other way, awaits further study.

Similar analysis of the data on nitrogen retention indicated that supplementing the soybean fatty acid diet with 0.035 g glucose/g fatty acids did not significantly ($P > 0.05$) increase nitrogen retention. The data indicated, however, that chicks fed the diet containing soybean oil utilized nitrogen more efficiently than did chicks fed comparable diets in which energy was supplied by soybean fatty acids with and without added glucose. In this experiment, the energy contents of the diets were calculated using the predetermined values of 3.64, 3.81, 9.21 and 8.65 kcal/g for the metabolizable energy content of glucose, soybean protein, soybean oil and soybean fatty acids, respectively. In three subsequent studies, the metabolizable energy content of soybean fatty acids has been found to vary, the values obtained being 7.54, 7.54

and 8.40 kcal/g. If, in this experiment, the energy value of soybean fatty acids was less than 8.65 kcal/g, chicks then would have consumed fewer non-protein calories than indicated and may have burned protein to meet their energy requirements. Whether this is the explanation for the difference in nitrogen retained by chicks fed soybean fatty acids and those fed soybean oil awaits further study.

Results showing caloric gain, caloric consumption and efficiency with which chicks utilized energy when pair-fed diets in which non-protein energy was supplied by soybean fatty acids, soybean fatty acids + 0.035 g glucose/g fatty acids and soybean oil are summarized in Table 11.

Table 11

Effect of glycerol and glucose on energy utilization when chicks are pair-fed.

Source of non-protein energy	Kcal consumed[1]	Energy gained, kcal		Kcal consumed /kcal gained
		Protein	Fat	
S.F.A.[2]	1717	261	160	4.07
	1598	264	111	4.26
	1658[3]	262	136	4.16
S.F.A. + 0.035 g glucose/g F.A.	1601	288	167	3.52
	1593	269	141	3.88
	1597	278	154	3.70
Soybean oil	1644	316	283	2.74
	1661	349	242	2.81
	1652	332	262	2.78

1. Calculated using calculated metabolizable energy values for the diets.
2. Soybean fatty acids.
3. Underlined values are averages of duplicate groups.

Analysis of variance (11) and application of Duncan's multiple range test (12) to the data on energy utilization indicated that the addition of 0.035 g glucose/g soybean fatty acids did not increase efficiency of utilization of energy ($P > 0.05$). Chicks fed the soybean oil containing diet, however, utilized energy more efficiently than did the other chicks ($P < 0.05$). Data on body composition showed that chicks fed soybean oil deposited not only more energy as protein but more as fat than did chicks fed either soybean fatty acids or soybean fatty acids + 0.035 g glucose/ g fatty acids.

EXPERIMENT 7

This experiment was conducted to confirm that the amount of protein retained by chicks fed "carbohydrate-and glycerol-free" diets is not increased by the addition of carbohydrate and to extend studies of the effect of carbohydrate on energy utilization in chicks pair-fed diets containing graded levels of glucose.

Materials and Methods

Duplicate groups of ten male crossbred chicks (White Plymouth Rock X Dominant White) were fed diets in which non-protein energy was supplied by soybean oil, soybean fatty acids and soybean fatty acids supplemented with 0.035 and 0.210 g glucose/g fatty acids. The nutrient intake of all chicks was limited to that consumed by chicks fed the diet containing soybean fatty acids with no added glucose.

The composition of the diets fed is given in Table 12. The diets were formulated to supply 14.3 kcal/g protein instead of 15.4 kcal/g

Table 12

Composition of diets

Treatment		Constant ingredients[2]	Soybean oil	S.F.A.	Glucose	Cellulose[3]	Total
Source of energy	Level of glucose g/g S.F.A.[1]	g	g	g	g	g	g
Soybean Oil	-	34.90	25.73	-	-	7.19	67.82
S.F.A.	-	34.90	-	31.43	-	7.19	73.52
"	0.035	34.90	-	30.80	1.09	7.19	73.98
"	0.210	34.90	-	28.01	5.96	7.19	76.06

1. Soybean fatty acids.

2. Constant ingredients supply in grams: 25.35 promine, 0.70 glycine, 0.90 methionine, 2.00 soybean oil, 1.49 $CaCO_3$, 1.70 $CaHPO_4$, 0.60 NaCl, 1.25 mineral mix, 0.59 vitamin mix, 0.025 antioxidant, 0.30 Cr_2O_3.

3. Solka Floc S.W. - 40-A, Brown Forest Products Ltd., Montreal, Quebec.

protein. Results have shown that the metabolizable energy content of soybean fatty acids is variable. Initially soybean fatty acids were found to have a metabolizable energy value of 8.65 kcal/g. In two later studies, however, the metabolizable energy value was found to be 7.54 kcal/g. Since the possibility existed that previous diets containing soybean fatty acids actually contained 14.3 kcal/g protein rather than 15.4 kcal/g protein, all diets in this experiment were formulated to contain 14.3 kcal/g protein.

The methods of housing, allotment and weighing were similar to those of Experiment 6 except that chicks were assigned to their respective groups at 7 rather than at 4 days of age. During the fourth week of the experiment, excreta were collected from each experimental group at 24 hour intervals on three successive days for the determination of metabolizable energy. Chromic oxide was incorporated in each of the diets at a level of approximately 0.3%, as an index substance, in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. The methods for processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy and chromic oxide and computing metabolizable energy from these data have been described previously. (Hill and Anderson, 20; Hill et al., 21).

At the termination of the experiment the chicks were killed without loss of blood. After cooling, the contents of the gastro-intestinal tracts were removed and the entire carcasses from each experimental group were frozen, ground, mixed and an aliquot dried by lyophilization. In order that tissue gains could be determined, a representative group of chicks was

killed at the beginning of the experiment and prepared for analysis in a similar fashion. Carcass samples were analyzed for protein, fat and moisture as described by Hill and Anderson (20).

Results and Discussion

Summarized in Table 13 are data showing growth and protein retention when chicks were pair-fed diets containing graded levels of glucose.

TABLE 13

Growth and protein retention when chicks are pair-fed diets containing graded levels of glucose

Treatment: Source of energy	Treatment: Level of glucose g/g S.F.A.[1]	Avg. wt. 4 wks g	Protein gain g	Protein intake g	Protein retention[2] %
S.F.A.	-	369	53.1	109.2	48.6
		373	56.0	109.7	51.0
		371[3]	54.6	109.4	49.8
"	0.035	378	53.4	103.2	51.7
		371	56.1	106.4	52.7
		374	54.8	104.8	52.2
"	0.210	373	56.5	109.6	51.5
		378	57.3	109.4	52.4
		376	56.9	109.5	52.0
Soybean Oil	-	374	56.0	104.8	53.4
		379	55.7	104.8	53.1
		376	55.8	104.8	53.2

1. Soybean fatty acids.
2. (Gain in carcass protein, g/protein consumed, g) X 100.
3. Underlined values are averages of duplicate groups.

The data showed that when the nutrient intake of chicks fed diets

containing soybean fatty acids plus glucose or soybean oil was limited to that of chicks fed soybean fatty acids, growth rates did not differ. These results are in agreement with results obtained in Experiment 6 and lend support to the concept that glucose added to a "carbohydrate-free" diet containing soybean fatty acids stimulated growth by increasing appetite.

Analysis of variance (11) and application of Duncan's multiple range test (12) to the data on nitrogen retention showed that chicks fed soybean fatty acids retained the same amount of nitrogen as did chicks pair-fed similar diets containing soybean oil or soybean fatty acids plus either 0.035 or 0.210 g glucose/g fatty acids. These results show either that soybean protein contains glucogenic amino acids in sufficient excess to meet the chick's requirement for glucose or that the chick can synthesize carbohydrate from fatty acids. Further studies using diets containing protein of a higher biological value or in which protein is replaced by a mixture of essential amino acids plus a source of non-protein nitrogen might be helpful in ascertaining the role which glucogenic amino acids play in enabling chicks to grow when fed "carbohydrate-and glycerol-free" diets.

The finding that the deletion of glycerol from a "carbohydrate-free" diet by substituting soybean fatty acids for soybean oil did not decrease nitrogen retention was in contrast to results of Experiment 6. In this experiment (Experiment 7), the amount of energy consumed by chicks fed diets containing soybean fatty acids was underestimated.

Analysis showed the metabolizable energy content of soybean fatty acids to be 8.40 rather than 7.54 kcal/gram. Whether the difference in caloric intake of chicks fed a "carbohydrate-free" diet containing soybean oil and those fed a comparable diet containing soybean fatty acids accounted for the difference between experiments is unknown and awaits further study. The data did show that the growth stimulating effect of glucose when added to a diet in which non-protein energy was supplied by soybean fatty acids was not due to its effect on nitrogen utilization.

Summarized in Table 14 are data showing caloric gain, caloric consumption and efficiency with which chicks utilized energy when pair-fed diets containing graded levels of glucose.

Analysis of variance (11) and application of Duncan's multiple range test (12) to the data on energy utilization showed that the deletion of glycerol from a "carbohydrate-free" diet by substituting soybean fatty acids for soybean oil decreased efficiency of utilization of energy ($P < 0.05$). The addition of 0.035 g glucose/g fatty acids did not increase energy utilization significantly ($P > 0.05$); however, when the level of supplementation was raised to 0.210 g glucose/g fatty acids, efficiency was increased ($P < 0.05$) but was still lower than when soybean oil was fed ($P < 0.05$). Similar results were obtained in Experiment 6 (Table 11) when non-protein energy was supplied by soybean fatty acids, soybean fatty acids + 0.035 g glucose/g fatty acids or soybean oil.

Data showing the amounts of energy deposited as fat and protein when feed intakes were restricted to that of chicks fed soybean fatty acids (Table 14) indicated that the addition of 0.210 g glucose/g fatty acids or

Table 14

Efficiency of utilization of energy by chicks pair-fed diets containing graded levels of glucose

Treatment					
Energy source	Level of glucose g/g S.F.A.[1]	Kcal consumed[2]	Energy gained, kcal Protein	Energy gained, kcal Fat	Kcal consumed / kcal gained
S.F.A.	-	1546	300	136	3.54
		1554	317	129	3.48
		1550[3]	314	132	3.51
"	0.035	1536	302	149	3.40
		1583	318	158	3.36
		1560	310	154	3.38
"	0.210	1570	320	176	3.17
		1568	324	160	3.21
		1569	322	168	3.19
Soybean Oil	-	1460	317	165	3.03
		1460	315	214	2.76
		1460	316	190	2.90

1. Soybean fatty acids
2. The metabolizable energy value for the diets was determined.
3. Underlined values are averages of duplicate groups.

the substitution of soybean oil for soybean fatty acids increased fat deposition. In Experiment 6 (Table 11) chicks fed "carbohydrate-free" diets containing soybean oil also deposited more energy as fat than did chicks fed comparable diets containing soybean fatty acids. Whether the reduction in fat synthesis when chicks were fed "carbohydrate-and glycerol-free" diets was due to lack of required factors for triglyceride synthesis e.g. glycerol, reduced triphosphopyridine nucleotide or whether the metabolic pathways used in the absence of carbohydrate and glycerol were less effective in their transfer of energy awaits further study.

PART III

METABOLIC EFFECTS OF FEEDING "CARBOHYDRATE-FREE" AND "CARBOHYDRATE-AND GLYCEROL-FREE" DIETS

Literature Review

Effect on Blood Glucose:

The blood sugar of a given species of animal is normally maintained within fairly narrow limits and at a level characteristic for that species. Sturkie (22) notes that, in the chicken, normal blood sugar values show considerable variation, with most values lying between 180 - 200 mg/100 ml blood. In contrast to the chick, the rat has a much lower level of blood glucose, ranging from 90 - 129 mg/100 ml (23). Altman (24) gives a fasting value for the rat of 87 mg/100 ml. Normal fasting values in the human range from 60 - 90 mg/100 ml (23)

The effect on blood glucose of feeding "carbohydrate-free" diets has not been studied in the chick. In the rat, Samuels et al. (25) found levels of blood glucose to be lowered significantly when "carbohydrate-free" diets were fed. In contrast, Kekwick and Pawan (26) and Azar and Bloom (8) observed that levels of blood glucose were not altered significantly when high fat, low carbohydrate diets were consumed by humans.

While studies of the metabolic effects of feeding "carbohydrate-free" diets are limited, information has accumulated on the metabolic effects of fasting when animals are burning their own tissue consisting

almost entirely of protein and fat. Burrows, Fritz and Titus (27) found that levels of blood sugar in male chickens dropped about 10% after fasting for 15 hours. Similar results have been reported by Golden and Long (28). They observed that level of blood sugar in chicks decreased about 10 mg/100ml during fasting. More recently Haupt (29) observed that blood sugar fell from about 200 mg to 190 and 170 mg/100ml in newly hatched and older chicks, respectively. In the case of the human and most other species, Keys (30) _et al_. have concluded that, during starvation, there is an initial decrease in blood sugar level followed by a gradual return to normal. They concluded that blood sugar levels were maintained close to control values during abstinence from food if the diets used before the fast were high in fat or protein.

Effect on Liver Glycogen:

In all species, level of liver glycogen reflects the intake of carbohydrate. Normal levels of liver glycogen in chicks (28), rats (23) and humans (23) have been reported to be 3%, 2.5 - 8.3% and 1.5 - 6.0%, respectively.

The effect on liver glycogen of feeding "carbohydrate-free" diets to humans and chicks has not been studied. Carr and Krantz (31) found level of liver glycogen to average 0.15% when rats were fasted for 48 hours and then fed a basal fat diet containing cacao butter for 72 hours. Lawrence and McCance (32) noted a drop in liver glycogen from 2.3% to 0.40% after a 24 hour fast. More recently, Mayes (33) found that level of liver glycogen decreased from 190 to 25 - 50 mg/100 g initial

body weight when rats were fed only butterfat, the extent being dependent on the amount of butterfat fed.

The decrease in liver glycogen due to fasting is dependent on the diet consumed prior to fasting (34). Mirski et al. (35) have shown that rats fed diets containing 70 and 90% casein retained liver glycogen much more tenaciously when starved for 48 hours than did rats fed diets containing 20% casein. Roberts, Samuels and Reinecke (36) and Stein et al. (34) showed that rats fed high fat, low carbohydrate diets retained more glycogen in their livers during fasting than did rats previously fed a high carbohydrate, low fat diet. Barbour (37) found levels of liver glycogen in rats fasted 24 and 48 hours to be 0.16% and 0.32% respectively. Prior to fasting the rats had been maintained on a high carbohydrate diet. Cori and Cori (38) confirmed the increase in glycogen content when the fast was extended from 24 to 48 hours. In the chicken, Golden and Long (28) reported that liver glycogen dropped from 3.0 to 0.4% during a 24 hour fast.

Effect on Blood Ketone Bodies:

Studies of the levels of ketone bodies in blood of chickens have not been reported. Normal levels of blood ketone bodies in rats and humans range from a trace to 2 mg/100 ml.

It is now well established that in the human and the rat, ketosis develops when fat metabolism is accelerated by feeding high fat, "carbohydrate-free" diets. Roberts and Samuels (39) found that blood ketone bodies increased from less than 1 mg/100 ml to 6.1 - 9.4 mg/100 ml

when adult male rats were force fed a high fat, low carbohydrate diet. Tepperman and Tepperman (40) also observed the blood ketone concentration of fat-fed rats to be significantly higher than that of carbohydrate-fed rats. In their experiments, however, substitution of fat for carbohydrate in the diet only increased ketone bodies from 1 to 3 mg/100 ml. In the case of the human, Azar and Bloom (8) recently reported that levels of blood ketones in normal adult humans increased from 0.47 to 3.3 mg/100 ml when high fat, low carbohydrate diets were consumed for 96 hours. Kekwick and Pawan (26) observed a more dramatic change; they reported that blood levels of ketone bodies increased from 1.9 to 30.5 mg/100 ml after three days when a normal subject was fed a diet containing 90% of the calories as fat in an amount to furnish 1000 kcal/day. In comparison they found that blood ketones increased from 2.0 to 4.1 mg/100 ml in obese humans subjected to the same dietary regime.

Ketosis has also been shown to develop in various species when carbohydrate metabolism is depressed, as in starvation. The degree to which ketosis develops during starvation in humans and rats is said to depend on sex, species, activity and the composition of the diet consumed prior to fasting. Tidwell and Treadwell (41) reported blood levels of ketone bodies in rats fasted 24 hours after receiving carbohydrate-containing and "carbohydrate-free" diets to be 7 mg/100 ml in both cases. In the human, Kartin (42) found the fasting level of ketone bodies in blood of 14 male subjects to be 0.4, 11.1 and 35.0 mg/100 ml after fasting 0, 2 and 6 days, respectively.

From the literature reviewed, it can be concluded that a limited number of experiments have been conducted to determine the effects of feeding high fat, low carbohyrate diets to humans and rats. Since no information is available on the metabolic effects of feeding "carbohydrate-free" diets to chicks, the following experiments were conducted to study levels of blood glucose, blood lactic acid, blood ketone bodies and liver glycogen in chicks fed "carbohydrate-free" diets in which non-protein calories were supplied by soybean oil and soybean fatty acids. For comparative purposes, studies also were conducted with chicks fed diets containing carbohydrate.

Materials and Methods

In the course of preceding experiments samples of blood and liver were taken from representative chicks at four weeks of age for the determination of blood glucose, blood lactic acid, blood ketone bodies and liver glycogen.

Blood samples were obtained by heart puncture using 3 mg potassium oxalate and 1 mg sodium fluoride per ml of blood to prevent coagulation and to inhibit glycolysis. Protein-free blood filtrates were prepared using barium hydroxide and zinc sulfate (43). The filtrates were frozen and stored at -17°C until analyzed.

Liver samples were taken immediately after killing chicks with sodium pentobarbital. The samples were frozen immediately using dry ice, wrapped in aluminum foil, and stored in plastic bags at -17°C until analyzed.

The method of Folin and Malmros (43) was used for the determination of blood glucose. Blood lactic acid was determined using the method of Barker and Summerson (43). Total blood ketone bodies were determined using a modification of the method of Bakker and White (44). This modification consisted of heating the tubes in an oil bath at 110 - 120^{o}C for 10 minutes and for a further 30 minutes after the addition of potassium dichromate. Liver glycogen was precipitated using the method of Good and co-workers (45). The precipitate was washed with 65% ethanol as suggested by Fong et al. (46). The glycogen was dissolved in water and estimated using the method of Seifter et al. (47).

Results and Discussion

Levels of blood glucose in chicks fed carbohydrate-containing and "carbohydrate-free" diets are summarized by the data in Table 15.

Analysis of variance (11) of the data in each of the experiments showed that neither level of dietary carbohydrate nor source of non-protein calories affected the level of blood glucose. These results indicated that chicks can maintain level of blood glucose in the absence of dietary carbohydrate and glycerol. Since chicks fed soybean fatty acids did not divert amino acids from protein to carbohydrate synthesis (Expt. 6 and 7) they must have derived glucose either from excess glucogenic amino acids in soybean protein or from fatty acids.

In the human, Kekwick and Pawan (26) and Azar and Bloom (8) showed that level of blood glucose was maintained when high fat, low carbohydrate diets were fed; however, in their experiments, an increased excretion of urinary nitrogen indicated that amino acids were

Table 15

Levels of blood glucose in chicks fed diets containing graded levels of glucose.

Treatment			Blood glucose mg/100 ml blood	
Source of energy	g/g S.F.A.[1] Glycerol	Glucose	Expt. 1[2]	Expt. 3[3]
S.F.A.	-	-	186[4] 208 <u>197</u>	182 180 <u>181</u>
"	0.108	-	225 238 <u>231</u>	-
"	-	0.035	-	210 179 <u>195</u>
"	-	0.105	-	182 179 <u>181</u>
"	-	0.210	-	266 226 <u>246</u>
Soybean Oil	-	-	210 210 <u>210</u>	-
Glucose	-	-	200 260 <u>230</u>	200 257 <u>229</u>

1. Soybean fatty acids.
2. Chicks fed experimental diets from 0 - 28 days of age (Renner, unpublished data).
3. Chicks fed experimental diets from 4 - 28 days of age (Expt. 3 - Table 6).
4. Values represent the average of duplicate determinations on 4 chicks. Underlined values are average values for duplicate groups.

being diverted from protein to synthesis of other compounds. In contrast to both the chick and the human, Mayes (33) and Samuels et al. (25) found that levels of blood glucose were lowered significantly when rats were fed high fat, "carbohydrate-free" diets.

Levels of lactic acid in blood of chicks fed diets with and without carbohydrate from hatching to 28 days of age are summarized by the data in Table 16.

Table 16

Levels of blood lactic acid in chicks fed diets with and without carbohydrate[1]

Source of non-protein calories	Lactic acid mg. /100 ml blood
S.F.A.[2]	27[3]
	47
	37
S.F.A. + 0.108 g glycerol /g S.F.A.	40
	42
	41
Soybean Oil	38
	26
	32
Glucose	27
	43
	35

1. Chicks fed experimental diets from 0 - 28 days of age (Renner, unpublished data).
2. Soybean fatty acids
3. Values represent the average of duplicate determinations on 4 chicks. Underlined values are average values for duplicate groups.

The results indicated the lactic acid content of blood of chicks fed diets in which non-protein energy was supplied by soybean fatty acids, soybean fatty acids + 0.108 g glycerol/g fatty acids, soybean oil or glucose to be variable and to be unaffected by source of non-protein energy. The results obtained are in reasonable agreement with values for non-laying chickens of 47 - 56 mg/100 ml blood as reported by Dukes (48). Nakatani and Gotoh (49) found that blood level of lactic acid in male chickens aged 80 - 90 days was not altered by fasting up to 72 hours.

Data showing the effect of source of non-protein energy on the glycogen content of liver are summarized in Table 17.

Table 17

Levels of liver glycogen in chicks fed diets with and without carbohydrate[1]

Source of non-protein calories	Liver glycogen % wet weight	
S. F. A.[2]	.04[3]	
	.17	
		.10
S.F.A. + 0.108 g glycerol g/S.F.A.	.46	
	.36	
		.41
Soybean oil	.83	
	1.03	
		.93
Glucose	1.96	
	2.64	
		2.28

1. Chicks fed experimental diets from 0 - 28 days of age (Renner, unpublished data).
2. Soybean fatty acids.
3. Values represent the average of duplicate determinations on 4 chicks. Underlined values are average values for duplicate groups.

Analysis of variance (11) and application of Duncan's multiple range test (12) showed that level of liver glycogen was reduced ($P < 0.05$) when calories from glucose were replaced by calories from soybean oil. The results also showed that level of liver glycogen was reduced still further ($P < 0.05$) when glycerol was deleted from the diet by substituting soybean fatty acids for soybean oil. In this experiment, the addition of glycerol in the amount required for theoretical conversion of fatty acids to triglyceride did not increase level of liver glycogen significantly ($P > 0.05$).

These results showed that gluconeogenesis is not of sufficient magnitude to maintain level of liver glycogen in the absence of dietary carbohydrate or dietary carbohydrate and glycerol. Carr and Krantz (31) and Mayes (33) have reported that level of liver glycogen was reduced in rats fed high fat, low carbohydrate diets. Fasting also has been reported to cause a marked reduction in liver glycogen in both rats (32) and chicks (28).

Summarized in Table 18 are results of determinations of ketone bodies on blood of chicks fed carbohydrate-containing and "carbohydrate-free" diets.

Analysis of variance (11) and application of Duncan's multiple range test (12) showed that deletion of carbohydrate from the diet by substituting soybean oil for glucose did not increase level of ketone bodies significantly ($P > 0.05$). Results showed, however, that levels of blood ketone bodies rose ($P < 0.05$) when glycerol was deleted from the

Table 18

Levels of blood ketone bodies in chicks fed diets with and without carbohydrate[1].

Source of non-protein calories	Blood ketone bodies mg/100ml blood[2]	
S.F.A.[3]	35.8[4]	
	28.5	
		32.2
S.F.A. + 0.108 g glycerol /g S.F.A.	12.8	
	11.5	
		12.2
Soybean oil	11.9	
	12.7	
		12.3
Glucose	4.4	
	5.2	
		4.8

1. Chicks fed experimental diets from 0 - 28 days of age (Renner, unpublished data).
2. Total ketone bodies as acetone.
3. Soybean fatty acids.
4. Values represent the average of duplicate determinations on 4 chicks. Underlined values are average values for duplicate groups.

"carbohydrate-free" diet by substituting soybean fatty acids for soybean oil. The addition of glycerol in the amount required for theoretical conversion of fatty acids to triglyceride reduced ketone bodies significantly ($P < 0.05$). These results indicated that gluconeogenesis was of sufficient magnitude to prevent the accumulation of ketone bodies in the absence of dietary carbohydrate but not in the absence of dietary carbohydrate and glycerol.

In a subsequent experiment, the effects on blood levels of ketone bodies of the additions of graded levels of glucose or protein to "carbohydrate-and glycerol-free" diets were studied. The results obtained, together with data on growth and caloric consumption from Tables 6 and 9 are summarized in Table 19.

In agreement with previous results, levels of blood ketone bodies increased when soybean fatty acids formed the sole source of non-protein energy in a diet containing 15.4 kcal/g protein. Analysis of variance (11) and application of Duncan's multiple range test (12) showed that the addition of protein in amounts to supply 13.2 and 11.0 kcal/g protein, rather than 15.4 kcal/g protein, reduced levels of blood ketones ($P < 0.05$). The data also showed that the addition of 0.035 g glucose/g fatty acids was as effective in preventing ketosis as the addition of 3 or 6 times that amount.

Correlation coefficients showed that levels of ketone bodies were negatively correlated ($P < 0.01$) with both caloric consumption and growth. Thus, elevated levels of blood ketones may be one factor which contributed to the reduction in appetite and growth in chicks fed "carbohydrate-and glycerol-free" diets.

Results of these studies (Table 18, 19 and 20) indicated average levels of blood ketone bodies in chicks fed diets in which non-protein calories were supplied by glucose or soybean oil to be 5.5 and 10.0 mg/100 ml, respectively. Comparable values for rats fed diets in which non-protein energy is supplied by either carbohydrate or triglyceride have

Table 19

Effect of the addition of graded levels of glucose and protein on growth, caloric consumption and blood levels of ketone bodies in chicks fed diets in which non-protein energy is supplied by soybean fatty acids.

Treatment					
Source of energy	Level of Protein kcal/g	Level of Glucose g/g S.F.A.[1]	Avg wt. 4 wks g	Caloric consumption kcal	Blood ketone bodies mg/100ml blood
Glucose	15.4	-	437[2]	2112[2]	5.7[3]
			457	2185	6.6
			447	2148	6.2
S.F.A.	"	-	326	1425	27.3
			343	1640	20.2
			335	1533	23.8
"	"	0.035	410	1850	6.4
			440	1980	7.5
			425	1915	6.9
"	"	0.105	424	1911	5.9
			377	1764	9.4
			400	1837	7.6
"	"	0.210	453	2112	4.4
			468	2222	4.8
			460	2167	4.6
"	13.2	-	362	1800	10.0
			372	1765	17.5
			367	1782	13.8
"	11.0	-	428	1968	7.5
			400	1848	12.9
			414	1908	10.2

1. Soybean fatty acids.
2. Values are averages of duplicate groups each containing 10 chicks.
3. Values represent the averages of duplicate determinations on 4 chicks. Underlined values are average values for duplicate groups.

been reported by Roberts and Samuels (39) to be < 1.0 and 6.1 - 9.4 mg/100 ml, respectively. Azar and Bloom (8) report values of 0.47 and 3.0 mg/100 ml, respectively, for humans fed diets with and without carbohydrate. These results suggest that levels of blood ketone in chicks fed diets containing carbohydrate are higher than in rats and humans receiving dietary carbohydrate and are approximately equal to values obtained in these species when "carbohydrate-free" diets are fed.

For comparative purpose fasting levels of blood ketone bodies were determined in fifteen 4-week old chicks that had been raised on a "carbohydrate-free" diet in which non-protein calories were supplied by soybean oil. Values obtained after fasting 0, 24, and 48 hours are summarized in Table 20.

Table 20

Levels of blood ketone bodies in chicks fasted for 24 and 48 hours

Hours fasted	Blood ketone bodies mg/100 ml blood	
0	7.75[1]	
	7.80	
	7.95	
		7.83
24	11.50	
	9.75	
	10.90	
		10.78
48	13.00	
	11.65	
	11.35	
		12.00

1. Values represent the average of duplicate determinations on 5 chicks. Underlined values are average values for triplicate groups.

Analysis of variance (11) showed that fasting for 24 or 48 hours significantly increased level of blood ketone bodies ($P < 0.05$). Results showed, however, that the increase in blood ketones brought about by fasting 24 or 48 hours was smaller in magnitude than the increase brought about by feeding a "carbohydrate-and glycerol-free" diet (Tables 18 and 19). This is to be expected since energy is stored as triglyceride and the glycerol contained therein has been shown (Table 18) to be sufficient to prevent accumulation of ketone bodies at least in the fed chick. Whether withholding feed from chicks fed "carbohydrate-and glycerol-free" diets would reduce levels of ketone bodies has not been determined.

GENERAL DISCUSSION

The ability of the chick to synthesize carbohydrate when fed "carbohydrate-free" diets containing soybean fatty acids is limited. Results have shown that, although gluconeogenesis is sufficient to maintain levels of blood glucose and blood lactic acid in the absence of dietary carbohydrate and glycerol, it is insufficient to promote optimum growth, to maintain level of liver glycogen or to prevent the accumulation of ketone bodies.

Results of pair-feeding studies showed that chicks fed "carbohydrate-free" diets containing soybean fatty acids utilized nitrogen as efficiently as chicks fed comparable diets supplemented with glucose (0.035 or 0.210 g glucose/g fatty acids). Although protein is a potential source of glucose results indicated that dietary protein was not diverted from protein to carbohydrate synthesis when "carbohydrate-free" diets containing soybean fatty acids were fed. This indicated either that soybean protein contains glucogenic amino acids in sufficient excess to maintain levels of blood glucose and blood lactic acid in the absence of dietary carbohydrate and glycerol or that chicks can synthesize carbohydrate from fatty acids.

Net conversion of fatty acids to carbohydrates in animals still remains in doubt. Recent studies have shown that a net synthesis of carbohydrate from fatty acids does occur in germinating high fat seeds (50) (51) and in certain microorganisms when they are grown in a medium in which acetate serves as the sole source of energy (52).

The reaction by which this net synthesis of carbohydrate is effected together with the enzymes involved may be summarized as follows:

$$\text{Isocitrate} \xrightarrow{\text{Isocitritase}} \text{Succinate} + \text{glyoxylate}$$

$$\text{Acetate} + \text{ATP} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{AMP} + \text{P-P}$$

$$\text{Glyoxylate} + \text{Acetyl CoA} \xrightarrow[\text{Synthetase}]{\text{Malate}} \text{Malate} + \text{CoA} \qquad (53)$$

Madsen (54), investigating the possibility of the glyoxylate by-pass in animal tissue, found that all tests for isocitritase and malate synthetase were negative and concluded that the glyoxylate cycle does not occur in animal tissues even where conversion of fat to carbohydrate is expected, e.g. in the developing chick embryo and in rats fed diets containing 95.4% butterfat for up to 38 days. More recently, Ganguli and Chakraverty (55) presented evidence for the presence of malic synthetase in liver and kidney tissue of the rat, guinea pig and rabbit. The presence of isocitritase has not yet been reported by this group.

The conversion of acetone to a three carbon intermediate of glycolysis has been shown in intact rats (56). Recently Mourkides, Hobbs and Koeppe (57) confirmed the existence of a pathway of acetone metabolism other than carboxylation to acetoacetate or cleavage to acetate and formate. They postulate a mechanism involving the direct conversion of acetone to a three carbon intermediate of glycolysis.

Whether these or other pathways for the conversion of fatty acids to carbohydrate are present in the chick is unknown. Since the

possibility exists that chicks fed "carbohydrate-and glycerol-free" diets may derive carbohydrate from excess glucogenic amino acids in soybean protein, studies might be directed towards reducing gluconeogenesis.

The reason why appetite is depressed when chicks are fed "carbohydrate-and glycerol-free" diets has not been determined. Blaxter (58) generalizes that, in specific dietary deficiencies, animals reduce the load on the enzyme systems affected by the deficiency. The primary method that animals employ is reducing food intake, but the enzyme system affected by the deficiency also may be avoided by using alternative metabolic pathways of dissimilation and synthesis, some of which are not as efficient in the transfer of energy. Results of my experiments suggest that chicks fed carbohydrate-deficient diets may use both of these mechanisms. Experiments (Exp. 1 - 4) showed that chicks fed "carbohydrate-free" diets containing soybean fatty acids consumed less feed and utilized it less efficiently than did chicks fed similar diets supplemented with glucose.

The enzyme systems which may be affected by carbohydrate deficiency have not been studied. Systems which may be involved and which may merit future study include those required for the synthesis of oxalacetic acid, α glycerophosphate and reduced triphosphopyridine nucleotide. A deficiency of these substances would favour fatty acid oxidation and the accumulation of ketone bodies.

The role which hormones play in facilitating a reduction in food intake in chicks fed carbohydrate-deficient diets is unknown.

Glucagon when administered intravenously has been shown to bring about hunger satiety in humans (59) and to inhibit gastric motility in both rats (60) and humans (59). Whether glucagon has similar effects when administered intravenously to chicks awaits further study.

Recently Unger _et_ _al_. (61) showed that level of circulating glucagon increased to three times the pre-starvation level when food was withheld from 3 male students for 72 hours. The question thus arises as to whether glucagon at this level brings about hunger satiety. Furthermore, does a carbohydrate deficiency in the chick result in an elevation of circulating glucagon and, if so, does it contribute to the reduction in food intake?

SUMMARY

1. A minimum level of dietary carbohydrate is required by the chick for normal growth and metabolism.
2. The amount of glycerol required for the maximum growth response of chicks fed diets calculated to contain 15.4 kcal/g protein, in which non-protein energy is supplied by soybean fatty acids, has been shown to be the amount required for theoretical conversion of fatty acids to triglycerides, i.e. 0.108 g glycerol/g fatty acids.
3. The amount of glucose required for the maximum growth response of chicks fed diets calculated to contain 15.4 kcal/g protein, in which non-protein energy is supplied by soybean fatty acids, has been found to vary from 1/3 to 1 times the amount required for theoretical conversion of fatty acids to triglyceride, i.e. 0.035 - 0.105 g glucose/g fatty acids.
4. The requirement of the chick for carbohydrate can be met by the addition of extra dietary protein. Results indicated that soybean protein was about one-tenth as effective as glucose in meeting this requirement.
5. Results of pair-feeding studies indicated that glucose increased growth of chicks fed "carbohydrate-free" diets containing soybean fatty acids by stimulating appetite.
6. Chicks fed "carbohydrate - free" diets containing soybean fatty acids utilized nitrogen as efficiently as chicks pair-fed comparable diets containing carbohydrate, i.e. 0.035 or 0.210 g glucose/

g fatty acids. The results indicated that dietary protein was not diverted from protein to carbohydrate synthesis when "carbohydrate-free" diets containing soybean fatty acids were fed.

7. Chicks fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids utilized energy less efficiently and deposited less carcass fat than chicks pair-fed comparable diets containing carbohydrate, i.e. 0.210 g glucose/g fatty acids.
8. Chicks fed "carbohydrate-free" diets in which non-protein calories were supplied by either soybean oil or soybean fatty acids maintained normal levels of blood glucose and blood lactic acid but showed a marked depression in level of liver glycogen. Further studies have shown that blood levels of ketone bodies rose when chicks were fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids but remained normal when soybean oil served as the sole source of non-protein energy.
9. Results of these experiments indicate that chicks fed "carbohydrate-and glycerol-free" diets containing soybean fatty acids derive carbohydrate for maintaining levels of blood glucose and blood lactic acid either from glucogenic amino acids in soybean protein or from fatty acids.

BIBLIOGRAPHY

1. Renner, R. 1964. Factors affecting the utilization of "carbohydrate-free" diets by the chick. I. Level of protein. J. Nutrition, 84: 322.
2. Renner, R. and A. M. Elcombe. 1964. Factors affecting the utilization of "carbohydrate-free" diets by the chick. II. Level of glycerol. J. Nutrition, 84: 327.
3. Osborne, T. B. and L. B. Mendel. 1924. Nutrition and growth on diets highly deficient or entirely lacking in preformed carbohydrates. J. Biol. Chem., 59: 13.
4. Levine, H. and A. H. Smith. 1927. Growth experiments on diets rich in fat. J. Biol. Chem., 72: 223.
5. Greisheimer, E.M. 1931. Glycogen and fat formation in rats on carbohydrate free diets. J. Nutrition, 4: 411.
6. MacKay, E. M., R.H. Barnes and H. O. Carne. 1941. The influence of a diet with a high protein content upon the appetite and deposition of fat. Am. J. Physiol., 135: 193.
7. McClellan, W.S. and E.F. DuBois. 1930. Prolonged meat diets with a study of kidney function and ketosis. J. Biol. Chem., 87: 651.
8. Azar, G.J. and W.L. Bloom. 1963. Similarities of carbohydrate deficiency and fasting. Arch. Int. Med., 112: 338.
9. Donaldson, W.E., G.F. Combs, G.L. Romoser and W.C. Supplee. 1956. Studies on energy levels in poultry rations. II. Tolerance of growing chicks to dietary fat. Poultry Sci., 36: 807.

10. Rand, N.T., H.M. Scott and F.A. Kummerow. 1958. Dietary fat in nutrition of the growing chick. Poultry Sci. 37: 1075.

11. Snedecor, G.W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa.

12. Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Company, Inc., New York.

13. Wolffberg, S. 1876. Z.f. The glucose equivalent of fed protein. Biol. 12: 266. (Nutrition Reviews 10: 17. 1952)

14. Cantarow, A. and B. Schepartz. 1957. Biochemistry, ed. 2. W.B. Saunders Company, Philadelphia.

15. Munro, H.N. 1951. Carbohydrate and fat as factors in protein utilization and metabolism. Physiol. Rev., 31: 449.

16. Thompson, W.S.T. and H.N. Munro. 1955. Relationship of carbohydrate metabolism to protein metabolism; effect of substituting fat for dietary carbohydrate. J. Nutrition, 56: 139.

17. Jull, M.A. 1930. Poultry husbandry, ed. 1. McGraw-Hill Book Company, New York.

18. Grau, C.R. 1947. The phenylalanine and tyrosine contents of chicks and eggs. J. Biol. Chem., 168: 485.

19. Donaldson, W.E. 1964. Adaptation of the chick to dietary energy source. J. Nutrition, 82: 115.

20. Hill, F.W. and D.L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutrition, 64: 587.

21. Hill, F. W., D. L. Anderson, R. Renner and L. B. Carew, Jr. 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poultry Sci., 39: 573.

22. Sturkie, P. D. 1954. Avian physiology. Comstock Publishing Company, Inc., Ithaca, New York.

23. Soskin, S. and R. Levine. 1960. The role of carbohydrates in the diet, p. 152. In Wohl, M. G. and R. S. Goodhart, Modern nutrition in health and disease, ed. 2. Lea and Febiger, Philadelphia.

24. Altman, P. L. 1961. Blood and other body fluids. A S D Technical Report 61 - 199. June 1961.

25. Samuels, L. T., R. C. Gilmore and R. M. Reinecke. 1948. Previous diet, fasting and work. J. Nutrition, 36: 639.

26. Kekwick, A. and G. L. S. Pawan. 1957. Metabolic studies in human obesity with isocaloric diets high in fat, carbohydrate or protein. Metabolism, 6: 477.

27. Burrows, W. H., J. C. Fritz and H. W. Titus. 1935. The blood sugar of the fasting, gizzardectomized fowl. J. Biol. Chem., 110: 39.

28. Golden, W. R. C. and C. N. H. Long. 1942. The influence of certain hormones on the carbohydrate levels of the chick. Endocrinol., 30: 675.

29. Haupt, T. R. 1958. Effects of fasting on blood sugar levels in baby chicks of varying ages. Poultry Sci., 37: 1452.

30. Keys, A. 1950. The Biology of Human Starvation, Vol. I. Univ. of Minn. Press.

31. Carr, C.J. and J.C. Krantz, Jr. 1942. Carbohydrate metabolism p.177. In J.Q. Griffith, Jr. and E.J. Farris, The rat in laboratory investigation, ed. 2. Lippincott Company.

32. Laurence, R.D. and R.A. McCance. 1931. Distribution of glycogen in the rat. Biochem. J., 25: 572.

33. Mayes, P.A. 1960. An inverse relation between the liver glycogen and the blood glucose in the rat adapted to a fat diet. Nature (London), 187: 325.

34. Stein, L., E. Tuerkischer and E. Wertheimer. 1939. The regulation of glyconeogenesis. J. Physiol., 95: 356.

35. Mirski, A., J. Rosenbaum, L. Stein and E. Wertheimer. 1938. On the behaviour of glycogen after diets rich in protein and in carbohydrate. J. Physiol., 92: 48.

36. Roberts, S., L.T. Samuels and R.M. Reinecke. 1944. Previous diets and the apparent utilization of fat in the absence of the liver. Am. J. Physiol., 140: 639.

37. Barbour, A.D. 1927. Influence of insulin on liver and muscle glycogen in the rat under varying nutritional conditions. Am. J. Physiol., 80: 246.

38. Cori, C.F. and C.T. Cori. 1928. The fate of sugar in the animal body. J. Biol. Chem., 76: 755.

39. Roberts, S. and L.T. Samuels. 1943. Fasting ketosis and nitrogen excretion as related to the fat content of the preceding diet. J. Biol. Chem., 151: 267.

40. Tepperman, H.M. and J. Tepperman. 1955. Ketogenesis in rats on high carbohydrate and high fat diets. Am. J. Physiol., 180: 511.

41. Tidwell, H.C. and C.R. Treadwell. 1946. The effect of the preceding diet upon fasting ketonemia. J. Biol. Chem., 162: 155.

42. Kartin, B.L., E.B. Man, A.W. Winkler and J.P. Peters. 1944. Blood ketones and serum lipids in starvation and water deprivation. J. Clin. Invest., 23: 824.

43. Hawk, P.H., B.L. Oser and W.H. Summerson. 1954. Practical Physiological Chemistry, ed. 13. The Blakiston Company, Inc., New York, pp. 543, 575, 622.

44. Bakker, N. and R. White. 1960. Simplified micromethod for the colorimetric determination of total acetone bodies in blood. New Zealand J. Sci. and Technol. 38: 1001. (Chem. Abstr. 54: 14345C)

45. Good, C.A., H. Kramer and M. Somogoyi. 1933. Determination of liver glycogen. J. Biol. Chem., 100: 485.

46. Fong, J., F.L. Schaffer and P.L. Kirk. 1953. The ultramicrodetermination of glycogen in liver. A comparison of the anthrone and reducing sugar methods. Arch. Biochem. Biophys., 45: 319.

47. Seifter, S., S. Dayton, B. Novic and E. Muntwyler. 1950. The estimation of glycogen with anthrone reagent. Arch. of Biochem., 25: 191.

48. Dukes, H.H. Physiology of domestic animals, ed. 5. Comstock Publishing Company, Inc., Ithaca, New York.

49. Nakatani, Y. and J. Gotoh. 1961. The effect of short-term fasting on blood glucose, lactic acid in blood, liver glycogen, muscle glycogen and body weight of the chicken. Jap. J. Zootech. Sci., 32: 119 (Nutr. Abs. and Rev. 32: 466.).

50. Kornberg, H.L. and H. Beevers. 1957. The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. Biochem. et Biophys. Acta, 26: 531.

51. Bradbeer, C. and P.K. Stumpf. 1959. Fat metabolism in higher plants: conversion of fat into carbohydrate in peanut and sunflower seedlings. J. Biol. Chem., 234: 498.

52. Kornberg, H.L. and H.A. Krebs. 1957. Synthesis of cell constituents from C_2 units of modified tricarboxylic acid cycle. Nature, 179: 988.

53. Kornberg, H.L. and N.B. Madsen. 1957. Synthesis of C_4 di-COOH acids from acetate by a "glyoxylate bypass" of TCA cycle. Biochem. et Biophys. Acta, 24: 651.

54. Madsen, N.B. 1958. No evidence for the glyoxylate by-pass in animal tissue. Biochem. et Biophys. Acta, 27: 199.

55. Ganguli, N.C. and K. Chakraverty. 1961. Evidence for malic synthetase in animal tissues. J. Am. Chem. Soc., 83: 2481.

56. Sakami, W. and J.M. Lafaye. 1951. The metabolism of acetone in the intact rat. J. Biol. Chem., 193: 199.

57. Mourkides, G.A., D.C. Hobbs and R.E. Koeppe. 1959. The metabolism of acetone$_2C^{14}$ by intact rats. J. Biol. Chem., 237: 27.

58. Blaxter, K. L. 1964. Dietary factors affecting energy utilization. Proc. Nut. Soc., 23: 3.

59. Stunkard, A. J., T. B. Van Itallie and B. B. Reis. 1955. The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. Proc. Soc. Exptl. Biol. and Med., 89: 258.

60. Salter, J. 1960. Metabolic effects of glucagon in the Wistar rat. Am. J. Clin. Nut., 8: 535.

61. Unger, R. H., A. M. Eisentraut and L. L. Madison. 1963. The effects of total starvation upon the levels of circulating glucagon and insulin in man. J. Clin. Invest., 42: 1031.